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TESTING THE MUTAGENIC POTENTIAL OF HE 1002 IN THE MOUSE LYMPHOMA MUTATION TEST (FINAL REPORT) WITH COVER LETTER DATED 112291

Chemical Category

4,4-DIPHENYLMETHANE DIISOCYANATE (101-68-8)

CONTAINS NO CBI

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Mobay

A Bayer USA INC COMPANY

Mobay Corporation
Health, Environment, Safety & Plant Management

Mobay Road
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November 22, 1991

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The information required at 40 CFR 716.30 is given below.

Chemical Name:	4,4-Diphenylmethane diisocyanate
CAS No:	101-68-8
Name of Study:	Testing the Mutagenic Potential of HE 1002 in the Mouse Lymphoma Mutation Test
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If you have any questions, please contact me.

Sincerely,

Francis J. Rattay
Manager, Regulatory Compliance
(412) 777-7471

Attachments
Certified Mail No.: P 276 377 332

R 2030

CONTAINS NO CBI 24408

TESTING THE MUTAGENIC POTENTIAL OF HE 1002 IN THE MOUSE LYMPHOMA MUTATION TEST

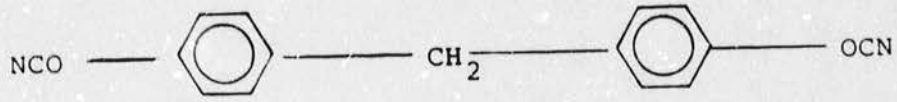
IRI Project No. 703542

IRI Inveresk Research International

Anhang zum Bericht:

Testing the mutagenic potential of HE 1002 in the mouse lymphoma mutation test.
IRI Project No. 703842

Bei HE 1002 handelt es sich um Desmodur 44 M. Desmodur 44 M (MDI) ist ein chemisch reiner Körper mit der Summenformel $C_{15}H_{10}O_2N_2$ und der Struktur



Die Studie trug die BAYER Studien Nr.:

DESMODUR 44 M / 014

Wuppertal, den 15. Juli 1981

(Dr. B. Herbold)
Monitor

INVERESK RESEARCH INTERNATIONAL
Report No. 1906

CONFIDENTIAL

TESTING THE MUTAGENIC POTENTIAL OF HE 1002 IN THE MOUSE LYMPHOMA MUTATION TEST

IRI Project No. 703842

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January 1981

AUTHENTICATION

"I, the undersigned, hereby declare that this work was performed under my supervision, according to the procedures herein described and that this report represents a true and accurate record of the results obtained."

18 W.J. Harris, B.Sc., Ph.D.
Principal Investigator

Project No. 703842 Report No. 1906

QUALITY ASSURANCE AUTHENTICATION

The execution of this type of short-term study is not individually inspected. The processes involved are inspected at intervals according to a pre-determined schedule.

The report has been audited by IRI Quality Assurance personnel according to the appropriate Standard Operating Procedure and is considered to describe the methods and procedures used in the study. The reported results accurately reflect the original data of the study.

IRI PROJECT NO. 703842 Report No. 1906

Signed Andrew Waddell Date 26th June 1981.

Quality Assurance Manager

QAU/017.

CONTENTS

PERSONNEL INVOLVED IN PROJECT 703842

Division Head:	D.B. McGregor, B.Sc., Ph.D.
Principal Investigator:	W.J. Harris, B.Sc., Ph.D.
Project Leader:	C.A. Ross, B.Sc., Ph.D.
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SUMMARY

The compound HE 1002 was tested for potential mutagenicity in the mouse lymphoma L5178Y specific locus mutation test. The primary criterion used for a significant positive effect in this test was a doubling of the trifluorothymidine mediated mutation frequency of the thymidine kinase locus, over that obtained in a solvent treated negative control group. The mutagenicity tests were conducted in the presence and absence of a post-mitochondrial supernatant fraction from the livers of adult, male rats treated with Aroclor 1254 and the co-factors required for mixed function oxidase activity (S-9 mix).

An initial toxicity test was carried out in the absence of S-9 mix and with a dose range of 0.1 µg/ml to 1000 µg/ml of HE 1002. The compound was found to be completely toxic to mouse lymphoma cells at or above 1000 µg/ml. The dose range for the first mutation test was selected at 2.5 µg/ml to 250 µg/ml.

Four mutation tests were carried out in the presence and absence of S-9 mix. In the absence of S-9 mix, compound HE 1002 showed no evidence of mutagenic activity. At highly toxic concentrations in the presence of S-9 mix, HE 1002 induced a mutagenic response usually at a single dose level. Some slight mutagenic activity exhibited by HE 1002 at lower concentrations in the first experiment carried out in the presence of S-9 mix was not observed in subsequent tests.

The pattern of activity is suggestive of mutagenic potential, but the criteria for concluding that HE 1002 is a mutagen were not fully satisfied.

INTRODUCTION

The mutagenicity of compound HE 1002 was examined in the L5178Y mouse lymphoma mutation test. This test measures mutation frequencies at a specific locus in cultured mammalian cells which provides a convenient experimental system for testing potential environmental mutagens. Advantageous characteristics of the system include an ability to grow in suspension culture, a short generation time and a cloning efficiency near 100% in a simple soft agar cloning medium.

The mouse lymphoma cells used in the assay were those of strain L5178Y rendered heterozygous at the normally diploid thymidine kinase (TK) locus. Mutation from $TK^{+/-}$ to $TK^{-/-}$ was measured using a modification of the method of Clive et al., (1972;1977). The growth of mutant colonies in soft agar containing trifluorothymidine, which is non-toxic to cells lacking thymidine kinase, indicated that mutation had occurred at the TK locus. A validation of this system has recently been published (Clive et al., 1979). This validation consisted of dose response data with 43 chemicals which represent weak and potent mutagens and carcinogens which are weakly or non-mutagenic in the Ames bacterial mutation assay. Several carcinogens which are negative or difficult to detect in the standard Ames assay are mutagenic in the mammalian cell system.

The tests described in this report were conducted at the Inveresk Gate Laboratories of Inveresk Research International. They were carried out between 19 August and 19 November 1980.

MATERIALS AND METHODS

Sterile procedures were used throughout preparation of materials and experimental methods.

Chemicals

The compound HE 1002 was received from Bayer A.G. on 15 August 1980. It was described as a cream flaky solid and kept at room temperature in a metal container.

The positive control substances used for the mutagenicity tests were ethyl methanesulphonate (EMS) and 2-acetylaminofluorene (AAF), both of which were obtained from Koch-Light Laboratories, Colnbrook, Buckinghamshire.

Thymidine and trifluorothymidine (TFT) came from Sigma Chemical Company Limited, Poole, Dorset. Hypoxanthine was obtained from PL Biochemicals, Milwaukee, Wisconsin, U.S.A., while methotrexate came from the Lederle Laboratories Division of Cyanamid of Great Britain, Gosport, Hampshire, U.K.

The polychlorinated biphenyl mixture Aroclor 1254 was received from Analabs Incorporated, Newhaven, Connecticut, U.S.A.

The vehicle control dimethylsulphoxide (DMSO) was obtained from BDH Chemicals Limited, Poole, England.

Cells

L5178Y mouse lymphoma cells heterozygous at the TK locus were obtained from D. Clive, Research Triangle Park, North Carolina, U.S.A.

Cell Growth and Maintenance

Fischer's medium (10x) and horse serum were obtained from Gibco Laboratories Limited, Paisley, while donor horse serum was obtained from Flow Laboratories Limited, Irvine, Scotland. The basic cell growth medium (F_0P) consisted of Fischer's medium supplemented with penicillin (100 units/ml), streptomycin (100 µg/ml), sodium bicarbonate (1.125 g/l), sodium pyruvate (2 mM), pluronic acid (0.5% w/v) and glutamine (2 mM).

The addition of horse serum (10% v/v) to F_0P gave $F_{10}P$ which was the medium used for all cell growth and culture maintenance.

The addition to F_0P of donor horse serum (20% v/v) and sodium pyruvate to 4 mM gave cloning medium (CM), the medium required for colony formation during experiments.

During routine culture maintenance, cells were grown in Nunclon Delta tissue culture flasks at 37°C and in an atmosphere of 5% CO_2:95% air (v/v). Cell density was calculated daily using a Neubauer haemocytometer and cultures diluted with $F_{10}P$ to a concentration of 3×10^5 cells/ml.

The Activation Mixture

Animals

Male Fischer rats weighing 250-300 g were injected once i.p. with Aroclor 1254 (diluted in corn oil to a concentration of 200 mg/ml) at a dosage of 500 mg/kg 5 days before they were killed. The animals were allowed drinking water continuously, but food was withheld 16 h before they were killed.

Preparation of the 9000 g Supernatant Fluid from Livers

Freshly killed animals were thoroughly swabbed with 70% alcohol, the abdomen opened and liver removed, taking special care not to cut into the gastrointestinal tract. The livers were collected in tared beakers containing ice-cold homogenisation medium. The medium used was 0.15 M-KCl.

The beakers were weighed and the collected livers transferred to the homogenisation vessel. A volume of ice-cold 0.15 M-KCl, equivalent to 3 times the weight of the liver was added to the vessel and the livers chopped using long handled scissors. The chopped livers were homogenised by 8 strokes of a glass tube vessel while the Teflon pestle (radial clearance 0.14-0.15 mm) was rotating at about 1200 r.p.m. The homogenate was transferred to sterile polypropylene centrifuge tubes and spun to give 9000 g for 10 min at $0^{\circ}C$-$+2^{\circ}C$. The supernatant fluid was decanted leaving behind a thick pellet of (mainly) whole cells, nuclei and mitochondria. Post-mitochondrial supernatant fluids were freshly prepared in sufficient quantity for this experiment.

Preparation of the "S-9 Mix"

Ice-cold 0.05 M phosphate buffer, pH 7.4, was added to preweighed NADP and glucose-6-phosphate, etc., as follows, to give a final concentration in the "S-9 mix" of:

NADP-di-Na salt	4 mM	(= 3.366 mg/ml)
Glucose-6-phosphate-di-Na salt	5 mM	(= 1.521 mg/ml)
$MgCl_2.6H_2O$	8 mM	(= 1.626 mg/ml)
KCl	33 mM	(= 2.460 mg/ml)

This solution was immediately filter sterilised by passage through 0.45 µm Millipore filter and mixed with the liver 9000 g supernatant fluid in the following proportion:

co-factor solution	9 parts
liver preparation	1 part

Toxicity Test

An initial toxicity test was carried out in the absence of S-9 activation in order to select doses of the chemical for mutation test.

10 ml samples of mouse lymphoma culture, containing 3×10^6 cells were exposed to one of 5 doses of compound.

Dilutions were carried out as follows:

		Final Concentration
(1)	Stock solution HE 1002 at 100 mg/ml in DMSO	1000 µg/ml
(2)	0.1 ml (1) +0.9 ml DMSO	100 µg/ml
(3)	0.1 ml (2) +0.9 ml DMSO	10 µg/ml
(4)	0.1 ml (3) +0.9 ml DMSO	1 µg/ml
(5)	0.1 ml (4) +0.9 ml DMSO	0.1 µg/ml

Incubation was carried out for 3 h at 37°C. After incubation, the cells were harvested by centrifugation at 1000 r.p.m. in the MSE Chilspin centrifuge for 5 min, then

resuspended in 10 ml $F_{10}P$. Each harvested culture was transferred to a 75 cm^2 Nunclon Delta tissue culture flask, gassed thoroughly with 5% CO_2:95% air (v/v) and left to grow at 37°C.

Cell density was measured by counting with a Neubauer haemocytometer each day for the next 3 days until the toxic effects of the chemical could be estimated.

Mutation Test

3×10^6 exponentially growing $TK^{+/-}$ mouse lymphoma L5178Y cells were dispensed to sterile, plastic universal bottles in 5 ml $F_{10}P$. 5 ml F_0P was added to give 10 ml of cells in F_5P medium.

The concentrations of test substance HE 1002 used in the mutation test were finally fixed at 250 µg/ml, 83 µg/ml, 25 µg/ml, 8.3 µg/ml and 2.5 µg/ml.

0.1 ml of appropriate test compound solution was added to each bottle, followed by 1 ml of freshly prepared S-9 mix if required. All bottles were incubated on a gyrotary shaker at 37°C, 150 r.p.m., for 3 h.

After the incubation period, the cells were harvested by centrifugation at 1000 r.p.m. for 5 min, then resuspended in 10 ml $F_{10}P$. A sample was taken from each of these cell suspensions and plated in soft agar to determine cell survival.

Survival Assay

A survival assay was carried out immediately after exposure of cells with test compound. Cells were diluted as follows:

(A) 0.1 ml cell suspension + 4.9 ml $F_{10}P$ medium
0.1 ml (A) + 21 ml cloning medium containing 0.25% agar.

7 ml samples were poured into each of 3 x 58 mm tissue culture Petri dishes and left to set at 4°C. After gelling, the plates were equilibrated with 5% CO_2:95% air (v/v) in plastic containers which were then sealed and incubated at 37°C for 10 days.

Expression of Genetic Damage

For 3 days after exposure to mutagen, cells were allowed to multiply in $F_{10}P$ medium. Cultures were counted each day using a Neubauer haemocytometer and diluted to 3 x 10^5 cells/ml.

On the third day after exposure, the cells were adjusted to 3 x 10^5 cells/ml and a sample taken for the survival assay for which it was diluted as follows:

(A) 0.1 ml cell suspension + 4.9 ml $F_{10}P$.
0.1 ml (A) + 21 ml cloning medium containing 0.25% agar.

The cloning medium suspension was then dispensed to 3 x 58 mm petri dishes and the plates processed as for the day 0 survival estimation.

For mutant colony selection in trifluorothymidine (TFT) cloning medium, 2 x 5 ml samples of suspension were dispensed into plastic universal bottles and the cells harvested by centrifugation. Each pellet was resuspended in 19.5 ml cloning medium without agar. Molten agar was added to give a concentration of 0.25% and the medium dispensed into 3 x 58 mm Petri dishes as for the survival assay. Hence, 6 TFT plates were prepared from each test culture.

After gelling and equilibration in a 95% air:5% CO_2 (v/v) atmosphere, the plates were sealed in boxes and incubated at 37°C. Colonies were counted manually 7-10 days later.

Calculations of Experimental Results

The number of cells plated per dish for the survival assay and for estimation of the number of TFT resistant mutants can be calculated from the figures given in the Experimental Methods.

For the survival assay, routinely 0.1 ml of 3×10^5 cells/ml + 4.9 ml $F_{10}P$ gave a 1:50 dilution.

0.1 ml of diluted culture of 6×10^3 cells/ml were added to 21 ml CM + agar and the suspension divided between 3 Petri dishes.

=) 200 cells were plated per dish.

For estimation of the number of TFT resistant mutants, 1.5×10^6 cells in total (5 ml of 3×10^5 cells/ml) were added to 21 ml CM and the suspension divided between 3 Petri dishes.

=) 5×10^5 cells were plated per dish.

If X is the average no. of survivors/plate.

Y is the average no. of TFT mutants/plate.

=) No. of survivors per ml of suspension after 3 days incubation $= (X) \times \frac{3 \times 10^5}{2 \times 10^2} = (X) \times 1,500$

=) No. of TFT mutants per ml of suspension after 3 days incubation $= (Y) \times \frac{3 \times 10^5}{5 \times 10^5} = (Y) \times 0.6$

=) No. of TFT mutants/10^5 survivors $= \frac{0.6Y}{1500X} \times 10^5$

If the numbers of cells plated in any particular experiment differed from those above, then the actual experiment figure has been quoted under the appropriate Table of Results.

RESULTS AND DISCUSSION

An initial toxicity test was carried out, in the absence of S-9 mix, and over the HE 1002 concentration range 0.1 µg/ml to 1000 µg/ml. At 1000 µg/ml, HE 1002 killed all the cells in the mouse lymphoma culture dosed with this concentration. All other cultures survived exposure to HE 1002, and the concentrations of test compound chosen for the first mutation test ranged from 2.5 µg/ml to 250 µg/ml.

The criterion used by Clive (Clive et al., 1979) to describe a positive result in this test was a doubling of the mutation frequency over the solvent treated control (spontaneous) value. In addition to this, a dose response shown by the induced mutation frequencies of at least 2 concentrations of test compound was considered necessary before a significant positive result was recorded.

The mutation frequencies obtained in these tests were all compared with the frequency observed in the solvent control group. In this laboratory, several dozens of mouse lymphoma mutation tests have been carried out during 1980. On only 2 occasions was the mutation frequency of the culture treated with DMSO greater than 10 mutants per 10^5 survivors. Mutation frequencies of between 4 to 9 mutants per 10^5 survivors were found in almost all other negative control cultures. The mutation frequency of 18 obtained for one of the DMSO-treated cultures in the first experiment in the absence of S-9 mix (Table 2) was, therefore, extremely unusual. This value was discounted when the results of the experiment were being evaluated.

Four mutation tests were carried out in both the absence and presence of S-9 mix. Tables 2 to 5 show the results of experiments carried out in the absence of S-9 mix. At 250 µg/ml, the top dose administered in the first mutation experiment

(Table 2), HE 1002 induced a mutation frequency greater than twice that of the negative control treated culture. Furthermore, there was a large number of colonies present on the TFT-containing Petri dishes. All other cultures treated with test compound in this experiment showed induced mutation frequencies similar to the negative control group values. The concentrations of HE 1002 administered to mouse lymphoma cultures in later experiments were, therefore, increased. Three subsequent experiments in the absence of S-9 mix (Tables 3 to 5) all produced no evidence for any mutagenic activity for HE 1002. The increased mutation frequency induced by 250 µg/ml test compound in the first of these tests (Table 2) was not repeated, either at higher concentrations of HE 1002 (Tables 3 and 4), or in the last experiment, carried out again over the concentration range 2.5 µg/ml to 250 µg/ml.

Tables 6 to 9 show the results of 4 mutation experiments carried out in the presence of S-9 mix. In the first experiment, all concentrations of HE 1002 induced a mutation frequency at least twice that occurring in the negative control culture (Table 6). At each concentration of test compound the absolute number of TFT^r mutants was also large compared to the number of mutant colonies produced by the DMSO-treated culture. In subsequent experiments, however, (Tables 7 to 9) this response was not observed, except for HE 1002 doses of 200 µg/ml or more.

In 3 out of 4 experiments in the presence of S-9 mix (Tables 6, 7 and 9) the mutation frequency induced by concentrations of HE 1002 > 200 µg/ml was very large indeed. On each occasion where a large mutation frequency was recorded, it had been noted that growth of the mouse lymphoma culture had been very slow. Thus, the mutagenic activity observed took place at concentrations of test compound producing conditions of high

toxicity (greater than 90%) in mouse lymphoma cultures. One reason for the slow growth of these cultures could be that cells were in contact with precipitated test compound both during the initial incubation of 3 h, and also during the subsequent 3 days of growth, precipitated material being harvested with the cells at the end of the scheduled 3 h exposure period. Growth, however, was not affected in cultures containing similar HE 1002 concentrations, but tested in the absence of S-9 mix. The S-9 mix, therefore, seems to generate metabolites which are more toxic and mutagenic than HE 1002 itself.

Relatively large numbers of TFT^+ mutant colonies were counted on Petri dishes containing cells from cultures dosed with >200 µg/ml HE 1002. Apart from the numbers of colonies recorded in the results tables, each Petri dish contained some hundreds of much smaller colonies. Several of these large and small colonies were individually removed from the plates, resuspended in Fischer's medium and allowed to grow to a density of 3×10^5/ml. When these liquid cultures were then treated with 1 µg/ml TFT, it was found that the cells from small colonies were sensitive to the chemical, whereas cells from large colonies were resistant. These smaller, non-mutant colonies were, therefore, omitted from the results calculations and their numbers do not appear in the Tables.

Table 8 shows the results of a mutation experiment carried out over the HE 1002 dose range of 12.5 µg/ml to 200 µg/ml in the presence of S-9 mix and where all concentrations of test compound induced mutation frequencies either the same or slightly greater than that from the negative control culture. On this occasion, the culture treated with 200 µg/ml test compound survived the exposure time enough to grow well during the subsequent 3-day mutation-fixation period. The reason why this culture behaved differently from the others is not known.

In conclusion, there is some evidence that compound HE 1002 shows mutagenic activity in this mutation test, in the presence of S-9 mix, but only under conditions of very high toxicity of the compound. Evidence of mutagenicity at lower HE 1002 concentrations produced in the first test carried out was not substantiated in later experiments. Furthermore, the criteria for a positive response were fully satisfied only in one experiment (Table 6); in the remaining experiments where significant responses were obtained, either a single dose level was involved (Tables 2 and 4) or the 2 significant doses were not consecutive (Table 7).

CONCLUSION

In the absence of S-9 mix, compound HE 1002 showed no evidence of mutagenic activity in this single locus mutation test. At highly toxic concentrations in the presence of S-9 mix, HE 1002 produced a clearly mutagenic response. The compound showed no mutagenic activity, however, at lower concentrations of 100 µg/ml and less.

REFERENCES

(1) Clive, D., Flamm, W.G., Machesko, M.R., and Bernheim N.J., (1972). A mutational assay system using the thymidine kinase locus in mouse lymphoma cells. Mutation Research, 16, 77-87.

(2) Clive, D. and Spector, J.F.S., (1977). Laboratory Procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Handbook of Mutagenicity Test Procedures, Kilbey et al., Eds. Elsevier Scientific Publishing Co., 161-173.

(3) Clive, D., Johnstone, K.O., Spector, J.F.S., Batson, A.G. and Brown, M.M.M. (1979). Validation and characterization of the L5178Y $TK^{+/-}$ mouse lymphoma mutagen assay system. Mutation Research, 59, 61-108.

TABLE 1

Cytotoxicity Test in the Absence of S-9

Project No.: 703842
Contractor: Bayer Study No. HE 1002/014
Operator: Carole Ross
Date of test: 19 August 1980

Substance: HE 1002
Activation: None
Liver preparation date: None
Cell culture batch: TK.2.8.5.

Substance quantity µg/ml	Haemocytometer Counts Total Visible Cells x 10^6		
	Day 1	Day 2	Day 3
HE 1001			
1000*	-	-	-
100*	6.8	27.0	90.0
10	7.7	31.5	139.0
1	8.2	29.7	78.0
0.1	6.2	23.2	62.4
DMSO	6.8	35.8	97.5
DMSO	9.5	34.7	85.8
DMSO	8.5	30.9	99.0

Selected dose range for mutation test:

250; 83; 25; 8.3; 2.5 µg/ml

*pptn of compound on contact with incubation medium.
pptn = precipitation

TABLE 2

Mutation Test - In the Absence of S-9
Plate Counts

Project No.: 703842
Contractor: Bayer Study No. HE 1002/014
Operator: Carole Ross
Date of test: 26 August 1980

Substance: HE 1002
Activation: None
Liver preparation date: None
Cell culture batch: TK 2.9.1.

Substance Quantity µg/ml	Day 0			Day 3				TFT^+ Colonies/ 10^5 Survivors
				Survival		TFT^+		
	Colonies/ dish	Average	Survival % control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1002								
250*	72, 59, 58	63	62	145,188, 165	166	107, 98, 66, 62, 89, 72	82	20
83*	157,132, 108	132	129	183,139, 196	173	44, 26, 17, 30, 32, 26	29	7
25	142,148, 115	135	133	160,243, 229	211	21, 27, 12, 22, 37, 40	27	5
8.3	132,123, 137	131	128	171,181, 208	187	18, 28, inf, 37, 29, 32	29	6
2.5	112,108, 113	111	109	204,203, 229	212	36, 26, inf, 36, 28, 30	31	6
EMS								
400	107, 95, 85	96	94	161,195, 184	180	147,178. 206,158, 224,160	180	40
200	96, 86, 107	96	94	217,232, 252	234	103,138, 122,109, 118,110	117	20
DMSO	60, 46, 51	52	51	180,163, 164	169	90, 95, 65, 76, 70, 66	77	18
DMSO	90,103, 113	102	100	163,227, 222	204	40, 24, 20, 21, 19, 18	25	5

*pptn of test compound on contact with incubation medium.
inf = infected

TABLE 3

Mutation Test - In the Absence of S-9
Plate Counts

Project No.: 703842
Contractor: Bayer Study No. HE 1002/014
Operator: Jennifer Liddle
Date of test: 22 September 1980

Substance: HE 1002
Activation: None
Liver preparation date: None
Cell culture batch: TK 2.8.2.

Substance Quantity µg/ml	Day 0			Day 3				TFT^+ Colonies/ 10^5 Survivors
				Survival		TFT^+		
	Colonies/ dish	Average	Survival % control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1002								
400*	75, 93, 72	80	98	214,266, 205	288	32, 22, 28, 23, 38, 31	29	5
200*	115, 91, 79	95	116	211,212, 253	225	21, 20, 26, 29, 30, 35	27	5
100	118,122, 101	114	139	193,165, 181	180	38, 42, 36, 44, 41, 38	40	9
50	74,113, 97	95	116	251,232, 248	244	43, 23, 31, 39, 28, 33	31	5
25	92,130, 104	109	133	223,254, 268	248	40, 52, 41, 37, 35, 30	41	7
EMS								
480	26, 29, 24	26	32	138,146, 153	146	188,156, 136,147, 152,147	154	42
240	42, 48, 55	48	59	141,170, 161	157	135,111, 86,131, 117,105	114	29
DMSO	78, 87, 82	82	100	204,219, 194	206	39, 50, 46, 32, 27, 34	38	7

*pptn of test compound on contact with incubation medium

TABLE 4

Mutation Test - In the Absence of S-9
Plate Counts

Project No.: 703842
Contractor: Bayer Study No. HE 1002/014
Operator: Carole Ross
Date of test: 21 October 1980

Substance: HE 1002
Activation: None
Liver preparation date: None
Cell culture batch: TK 2.4.2.1

Substance Quantity µg/ml	Day 0			Day 3				TFT+ Colonies/ 10^5 Survivors
				Survival		TFT+		
	Colonies/ dish	Average	Survival % control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1002								
400*	132, 90, 107	110	134	165,157, 164	162	37, 25, 20, 57, 21, 23	31	8
200	91,101, 102	98	120	203,234, 249	229	25, 27, 32, 32, 25, 21	27	5
100	96, 84, 87	89	109	186,225, 195	202	inf,inf, inf, 31, 35, 40	35	7
50	65, 75, 47	62	76	136,164, 132	144	37, 49, 45, 42, 45, 65	47	13
25	89,100, 88	92	112	119,152, 127	133	20, 9, inf, 18, 18, 17	16	5
EMS								
400	35, 46 46	42	51	184,135, 149	156	inf,inf, inf,157, 130,128	138	35
200	70, 59, 69	66	80	287,275, 249	270	121,112, 99,inf, 107, 93	106	16
DMSO	74, 76 inf.	75	82 = 100	247,244, 212	234	25, 26 inf, 24, 25, 23	25	4
DMSO	96, 92 76	88		177,172, 152	167	17, 18, 21, 36, 18, 30	23	6

*pptn of compound on contact with incubation medium.

pptn = precipitation

inf = infected

TABLE 5

Mutation Test - In the Absence of S-9
Plate Counts

Project No.: 703842
Contractor: Bayer Study No. HE 1002/014
Operator: Carole Ross
Date of test: 4 November 1980

Substance: HE 1002
Activation: none
Liver preparation date: none
Cell culture batch: TK 2.4.2.2

Substance Quantity µg/ml	Day 0			Day 3				TFT^+ Colonies/ 10^5 Survivors
				Survival		TFT^+		
	Colonies/ dish	Average	Survival % control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1002								
250*	106,108, 116	110	110	197,162, inf	180	42, 52, 54, 37, 52, 39	46	10
83*	115,110, 107	111	111	210,217, 231	219	48, 41, 56, 50, 57, 47	50	9
25	140,129, 138	136	136	192,184, 203	193	37,inf, 32, 28, 47, 48	38	8
8.3	143,124, 155	141	141	223,273, 303	266	53, 73, 58, 54, 62, 57	60	9
2.5	138,145, 174	152	152	123,203, 205	177	48, 46, 57, 58, 58, 54	54	12
EMS								
400	98, 86, 80	88	88	144,128, 159	144	140,180, 153,187, 150,135	158	44
200	120, 98, 104	107	107	209,177, 178	188	95,119, 96, 96, 95, 99	100	21
DMSO	95, 87, 118	100	100	244,220, 183	216	27, 33, 44, 42, 39, 45	38	7

*pptn of compound on contact with incubation medium
inf = infected

TABLE 6

Mutation Test - In the Presence of S-9
Plate Counts

Project No.: 703842
Contractor: Bayer Study No. HE 1002/014
Operator: Carole Ross
Date of test: 26 August 1980

Substance: HE 1002
Activation: Aroclor-induced Fischer Rat
Liver preparation date: 13 August 1980
Cell culture batch: TK 2.9.1.

Substance Quantity µg/ml	Day 0			Day 3				TFT^+ Colonies/ 10^5 Survivors
				Survival		TFT^+		
	Colonies/ dish	Average	Survival % control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1002								
250*	28, 20, 19	22	21	9, 8, 7	8	91, 63, 25, 49, 51, 83	60	300
83*	90, 90, 91	91	88	179,134, 159	157	80, 75 51, 62, 72, 47	65	17
25	97,109, 95	100	96	186,209, 263	219	66, 40, 63, 61, 52, 67	58	11
8.3	121,100, 141	121	116	186,224, 204	205	36, 35, 48, 33, 39, 40	39	8
2.5	109, 93, 93	93	94	205, 194 164	188	58, 64, 65, 67, 69, 43	61	13
AAF								
100	28, 31, 19	26	25	104, 97 89	97	69, 30, 48, 64, 18, 30	43	18
50	38, 46, 28	37	36	128,112, 153	131	87, 52, 76, 89, 61, 72	73	22
DMSO	98, 86, 91	92	104 = 100	212,inf, inf	212	1, 24, 37, 24, 14, 19	20	4
DMEO	98,138, 110	115		220,284, 144	216	15, 19, 20, 19, 22, 13	18	3

*pptn of test compound on contact with incubation medium.

inf = infected

TABLE 7

Mutation Test - In the Presence of S-9
Plate Counts

Project No.: 703842
Contractor: Bayer Study No. HE 1002/014
Operator: Carole Ross
Date of test: 22 September 1980

Substance: HE 1002
Activation: Aroclor-induced Fischer Rat
Liver preparation date: 13 August 1980
Cell culture batch: TK 2.8.2.

Substance Quantity µg/ml	Day 0			Day 3				TFT$^+$ Colonies/ 10^5 Survivors
				Survival		TFT$^+$		
	Colonies/ dish	Average	Survival % control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1002								
200*	101, 99, 87	96	58	1, 1, 1	1	82, 87, 113, 77, 72, 67	83	3320
100*	132,164, 147	148	90	152,171, 145	156	16, 20, 20, 12, 14, 23	18	5
50	140,164, 123	142	86	51, 52, 50	51	18, 18, 18,inf, inf,inf	18	14
25	153,145, 164	154	93	169,189, 161	173	20, 11, 22,inf, inf,inf	18	4
12.5	147,158, 149	151	92	236,231, 207	225	27, 28, 27, 33, 33, 34	30	5
AAF								
100	26, 16, 16	19	12	146,150, 138	145	68, 59, 44, 57, 55, 47	55	15
50	32, 57, 47	45	27	182,162, 184	176	61, 45, 39, 50, 48, 62	51	12
DMSO	175,153, 166	165	100	209,261, 219	230	27, 34, 31, 41, 42, 37	35	6

*pptn of test compound on contact with incubation medium.
inf = infected

TABLE 8

Mutation Test - In the Presence of S-9
Plate Counts

Project No.: 703842
Contractor: Bayer Study No. HE 1002/014
Operator: Carole Ross
Date of test: 21 October 1980

Substance: HE 1002
Activation: Aroclor-induced Fischer Rat
Liver preparation date: 13 August 1980
Cell culture batch: TK 2.4.2.1.

Substance Quantity µg/ml	Day 0			Day 3				TFT+ Colonies/ 10^5 Survivors
				Survival		TFT+		
	Colonies/ dish	Average	Survival % control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1002								
200*	147,100, 83	110	91	216,222, 212	217	39, 41, 45, 51, 31, 33	40	7
100*	50, 64, 38	51	42	209,166, 194	190	46, 48 38, 39, 49,inf	44	9
50	96,118, 94	103	85	167,198, 222	196	21, 29, 23, 23, 23, 15	22	4
25	129,142 105	125	103	180,190, 178	183	30, 30, 32, 25, 27, 37	30	7
12.5	113,122, 91	109	90	172,158, 157	162	22, 20, 24, 27, 29, 28	25	6
AAF								
100	2, 2, 0	1	0.008	80, 50, 50	60	45, 40, 39, 45, 43, 41	42	28
50	0, 0, 3	1	1	110,121, inf	116	97, 90, 110, 90, 71, 69	88	30
DMSO	102,108 96	102	121 ≈100	269,273, 174	239	27, 28, 31, 38, 26, 35	31	5
DMSO	162,140, 115	139		189,191, 204	195	24, 26, 28, 28, 43, 21	28	6

*pptn of test compound on contact with incubation medium.
inf = infected

TABLE 9

Mutation Test - In the Presence of S-9
Plate Counts

Project No.: 703842
Contractor: Bayer Study No. HE 1002/014
Operator: Carole Ross
Date of test: 4 November 1980

Substance: HE 1002
Activation: Aroclor-induced Fischer Rat
Liver preparation date: 13 August 1980
Cell culture batch: TK 2.4.2.2.

Substance Quantity µg/ml	Day 0			Day 3				TFT$^+$ Colonies/ 10^5 Survivors
				Survival		TFT$^+$		
	Colonies/ dish	Average	Survival % control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1002								
250*	94, 91, 80	88	82	27, 21, 18	22	50, 58, 52, 47, 49, 62	53	96
83*	87,111, 120	106	99	209,171, 184	188	39, 39, 32, 39, 46, 54	42	9
25	111,116, 143	123	115	213,237, 212	221	32, 36, 37, 67, 56, 59	48	9
8.3	109,131, 144	128	120	171,160, 202	178	28, 21, 26, 43, 39, 14	29	7
2.5	143,140, 186	156	146	180,199, 216	198	inf,inf, inf,inf, inf, 34	34	7
AAF								
100	79, 72, 73	75	70	149,174, 169	164	65, 71, 87, 79, 70, 87	77	19
50	127, 97, 95	106	99	175,184, 134	164	102,inf, 99, 88, 106, 94	98	24
DMSO	85,116, 119	107	100	124,129, 128	127	49, 19, 39, 36, 31, 34	35	11

*pptn of test compound on contact with incubation medium.
inf = infected

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